REMARKS

Applicant has amended claims 32, 45 and 57 to recite that the cells are examined by ^{19}F MRI. Support for the amendment can be found, for example, at page 5, lines 7-11.

Claims 32, 45, 57 and 69-88 are pending. These amendments do not add any new matter.

DETAILED ACTION

Applicant notes with appreciation that the amendment filed 5 January 2011 has been entered.

Election/Restrictions

Applicant notes with appreciation that the Examiner has rejoined Group IV to elected Group III.

Claim Rejections under 35 U.S.C. § 103

Claims 32, 45, 57, 69-77, 81-82 and 86-88 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Phillips (US 4,935,223) in view of Walters (US 5,460,800) and Lanza (US 5,690,907). The Examiner states that Phillips discloses a process for labeling of viable eukaryotic cells, administering the labeled cells to a patient and monitoring the localization of the labeled material. Phillips allegedly further discloses imaging techniques including magnetic resonance imaging. Walters allegedly discloses a method for labeling and visualizing cells and tissue by administering a composition comprising a physiologically acceptable fluorocarbon liquid. Walters also allegedly discloses that polyethers are encompassed within the broad definition of "fluorocarbon" materials. Lanza allegedly discloses that the lipid encapsulated particles constituted a perfluorocarbon emulsion. The Examiner states that it would have been obvious to one of ordinary skill in the art at the time the invention was made to incorporate fluorocarbon imaging agent into the viable cells as taught by Phillips. Applicant respectfully traverses.

As an initial point, Applicant notes that the instant claims have been amended to more particularly point out the claimed subject matter. Applicant's amendment is not in acquiescence of the rejection, and Applicant reserves the right to prosecute claims of similar or differing scope. The instant claims are directed to *ex vivo* labeled cellular formulations and methods for

detecting a cell in a subject, by administering to the subject a cell labeled with a fluorocarbon imaging reagent, and then examining at least a portion of the subject by ^{19}F magnetic resonance imaging (MRI), thereby detecting the fluorocarbon-labeled cell in the subject. None of the cited references teach or suggest pre-labeling cells with a fluorocarbon imaging reagent for administering to a subject and examining by ^{19}F MRI. Thus, as an initial point, Applicant notes that the combined teachings of the cited references fail to undermine the patentability of the claimed invention for, at least, failing to teach or suggest each and every element of the claimed invention. This and other arguments are discussed in more detail below.

I. The Cited References Fail to Teach or Suggest Each and Every Element of the Claimed Invention.

None of the cited references — neither Phillips, Walters nor Lanza — teach or suggest visualizing fluorocarbon imaging reagents by ^{19}F MRI. Thus, the combined teachings of the cited references fail to teach or suggest the claimed invention. Accordingly, the combined teachings of the cited references fail to render the claimed invention obvious.

^{19}F is an unconventional nucleus to image *in vivo* with MRI, as essentially no natural, MRI-detectable ^{19}F is present in the body. Thus, absent some specific teaching or suggestion present in the cited references, there is no motivation for one of skill in the art to select ^{19}F for use in the claimed methods. Simply put, there is no such teaching or suggestion in the cited references. Phillips is silent with respect to fluorocarbon imaging reagents. Moreover, one of skill in the art at the priority date of the instant application would not have thought that the electroporation methods of Philips would have been operative with a ^{19}F fluorocarbon imaging reagent as discussed in more detail below. Walters does not teach or suggest imaging fluorocarbons generally, or those detectable by ^{19}F MRI specifically. Rather, Walters teaches away from using magnetic resonance imaging for the detection of fluorocarbons by instead teaching detection of a visible or fluorescent dye (column 2, lines 8-14). Similarly, Lanza does not remedy the stated deficiencies, but instead teaches methods for ultrasonic imaging not methods for MRI imaging. As this review of the cited references illustrates, there is simply no teaching or suggestion regarding ^{19}F and no teaching or suggestion that would motivate one of skill in the art to select imaging reagents detectable by ^{19}F MRI. Since the cited art fails to teach

or suggest every element of the claimed invention, the combination of references fails to render the claimed invention obvious.

II. There is No Motivation for One of Skill in the Art to Combine the Cited References.

One of ordinary skill in the art would not have expected the Phillips method -- labeling cells with radionuclides or paramagnetic agents by electroporation -- to have been suitable for use with the instantly claimed fluorocarbon imaging reagents. Indeed, Phillips is completely silent with respect to fluorocarbon imaging reagents. Fluorocarbon imaging reagents are very different from radionuclides and paramagnetic agents in both their structure and their chemical properties. The following is exemplary of the fundamental differences between radionuclides and parametric agents versus fluorocarbon imaging reagents. Applicant teaches that fluorocarbon imaging reagents are formulated as emulsions to label cells (see page 36, line 29 – page 37, line 3). However, although electroporation is taught by Phillips as the method for labeling cells with radionuclides or paramagnetic agents, electroporation of emulsion particles, which are at least two orders of magnitude larger than particles described in Phillips, was only recently shown to be effective. *See* Srinivas et al., (2007) Magnetic Resonance in Medicine 58:725–734, Figure 2c, attached. Therefore, at the priority date of the instant application, one of ordinary skill in the art would not have been motivated to use the electroporation labeling method of Phillips with fluorocarbon imaging reagents.

Nor does Walters teach or suggest the claimed methods or formulations. Walters does not, in contrast to applicant, teach or suggest *ex vivo* labeling of cells with a fluorocarbon imaging reagent so that the labeled cells can be subsequently administered to a subject for imaging. In fact, Walters does not teach or suggest administration of labeled cells to a subject. Walters discloses only two methods: either "labeling in cell culture" for use and examination *in vitro* or directly injecting imaging reagents into a subject "in vivo" (column 3, lines 59-60). In other words, according to Walters, cells are either labeled and studied in cell culture, or imaging reagent is injected directly into a subject. In referring to "labeling in cell culture" at column 3, Walters does not refer to labeling of cells in tissue culture for subsequent *in vivo* use, but merely labeling of cells *in vitro* for *in vitro* purposes. A close reading of Walters shows that, when referring to *in vivo* labeling, the document is describing direct injection of a perfluorocarbon plasma mixture into an animal. There is no teaching or suggestion to administer ***any*** cells to a

patient – and certainly no teaching or suggestion to administer cells that were first labeled *ex vivo*. Thus, at the priority date of the instant application, the ordinarily skilled worker would not have read Walters to have taught or suggested *in vivo* administration of labeled cells, such as cells that had been labeled *ex vivo* prior to administration.

Similarly, Lanza does not teach or suggest labeling cells *ex vivo*. Rather, Lanza discloses *in vitro* cell culture labeling or directly injecting imaging reagents into a subject. In contrast to the teachings of Walters and Lanza, the instant claims are not directed to methods for direct injection of fluorocarbons into a subject. Rather, claim 1 (and claims depending therefrom) is directed to a method of detecting an *ex vivo* labeled cell by ^{19}F MRI in a subject, and claim 45 is directed to an *ex vivo* labeled cellular formulation. Accordingly, Lanza does not teach or suggest the instant claims.

Nothing in the disclosure of Walters or Lanza would have motivated one of ordinary skill in the art to use a perfluorocarbon imaging reagent generally, or to specifically use one detectable by ^{19}F MRI specifically, with the Phillips method. Neither Walters nor Lanza teach or suggest labeling cell *ex vivo* for *in vivo* use. Moreover, the ordinarily skilled worker would not have known whether a fluorocarbon imaging reagent exposed to a cell *ex vivo* would have remained stably associated with that cell *in vivo*. The direct injection and *in vitro* methods of Walters and Lanza do not teach or suggest that perfluorocarbon imaging reagents would be stably associated with a specific cell *in vivo*. In other words, there was no reason to think that perfluorocarbon imaging reagents would be useful in methods for administering pre-labeled cells *in vivo*. Furthermore, the compositions of Walters and Lanza were not known to be safe and effective for subsequent administration of *ex vivo* labeled cells until the instant disclosure. The concentration of fluorocarbon and duration of exposure to a cell are different between *ex vivo* labeling and direct injection into a subject. One of ordinary skill in the art would not have expected fluorocarbon imaging reagents to be suitable for *ex vivo* labeling simply because they are suitable for direct injection. Thus, one of ordinary skill in the art would not have been motivated to combine the perfluorocarbon imaging reagents of Walters or Lanza with the method of Phillips.

III. The Claimed Invention Produces Unexpected and Superior Results

Not only do the cited references fail to teach or suggest every element of the claimed invention, but the presently claimed invention also yields unexpected and superior results over the cited art. Applicant notes that, given that the prima facie case is not met for the cited references, Applicant has no obligation to present evidence of unexpected and/or superior results. Nevertheless, to help expedite prosecution, Applicant provides below a brief discussion of the unexpected and superior results of the claimed invention.

The claimed methods and formulations are effective for large-scale cell labeling applications. Such large-scale cell labeling would be impractical and ineffective using the electroporation method of Phillips, which is limited to the small number of cells that can be electroporated.

The claimed methods are also superior for clinical *ex vivo* applications. Such clinical applications require the survival and functionality of labeled cells because valuable primary cells from a patient are being labeled for administration back into the patient. Electroporation is not suitable for such applications because of its known detrimental secondary effects, such as cell lysis, in some cells (Tsong, (1991) Biophys J. 60(2):297-306, attached). Furthermore, Phillips does not examine any phenotype or functionality of cells labeled by this method to verify that the cells have not been detrimentally affected, which is critical for clinical applications. In fact, the radionuclide imaging reagents of Phillips, such as technetium, are known to be toxic to cells (see column 2, lines 25-30 of Phillips). Applicant's *ex vivo* fluorocarbon labeling, on the other hand, has no apparent toxicity or effect on proliferation of cells (page 42, line 20 – page 43, line 27). Applicant has also demonstrated that cells retain their normal phenotype and function after *ex vivo* fluorocarbon labeling (page 40, lines 5-22). As toxicity and functionality of cells had not previously been studied for *ex vivo* fluorocarbon labeling, the ordinarily skilled worker would not have expected that the claimed methods and compositions would be superior for large scale and clinical use.

A further unexpected superior property of *ex vivo* fluorocarbon labeling over the cited art is the extended retention of the fluorocarbon imaging reagent in cells. The Phillips' radionuclide imaging reagents, such as technetium, are known to leach out of cells over a short period of time (see column 2, lines 47-58 of Phillips). In contrast, applicant has demonstrated that fluorocarbon

imaging reagents result in little or no degradation or excretion in cells (page 41, line 18 – page 42, line 4). Therefore, the claimed methods and compositions allow for monitoring and tracking of labeled cells for significantly longer periods than could have been possible using the techniques of the cited art. A durable label to track cells *in vivo* over time is of great importance in certain applications in the field of cell therapy, for example. The benefits obtained by the presently claimed methods could not have been expected by one of ordinary skill in the art.

For all of the foregoing reasons, Applicant submits that the present claims are not rendered obvious by the teachings of Phillips in view of Walters and Lanza. The cited references fail to teach or suggest each and every element of the claimed invention. There is no motivation to combine the teachings of the cited references in an attempt to arrive at the claimed invention. Finally, the claimed invention provides unexpected and superior benefits over the cited art. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) are respectfully requested.

Claims 32, 78-80 and 83-85 are rejected under 35 U.S.C. § 103(a) as allegedly being unpatentable over Phillips (US 4,935,223) in view of Wilhelm (Eur. Biophys. J 31, 118-125, 2002) and Schweighardt (US 5,196,348). The Examiner states that Wilhelm discloses a method to quantify the uptake of magnetic nanoparticles in biological cells, such as dendritic cells. The Examiner states that Schweighardt discloses a method and compositions for improving magnetic resonance spectra of body organs and tissues using fluorocarbons such as perfluoro-15-crown-5-ether having enhanced signal to noise response ratios. The Examiner states that it would have been obvious to one of ordinary skill in the art at the time the invention was made to incorporate perfluoro-15-crown-5-ether into Phillips' method of labeling cells. Applicant respectfully traverses.

The ordinarily skilled worker would not have been motivated to substitute Schweighardt's perfluoro-15-crown-5-ether into Phillips' method of labeling cells as the Examiner suggests. As discussed *supra*, Phillips does not teach or suggest fluorocarbon imaging reagents or the use of ^{19}F MRI. Furthermore, one of ordinary skill in the art would not have expected the electroporation labeling method of Phillips to have been suitable for use with

fluorocarbon imaging reagents. In addition, the unexpected superior results of the claimed methods and compositions over Phillips, as described above, apply also to this rejection.

Nothing in the disclosure of Schweighardt would have motivated one of ordinary skill in the art to use a perfluoro-15-crown-5-ether with the Phillips method. Schweighardt relates to injecting perfluoro-15-crown-5-ether into a subject for determining oxygen levels in organs and body cavities rather than tracking cells. Labeling cells *ex vivo* is not taught or suggested by Schweighardt. Moreover, the ordinarily skilled worker would not have known whether a fluorocarbon imaging reagent exposed to a cell *ex vivo* would have remained stably associated with that cell *in vivo*. The direct injection methods of Schweighardt do not teach or suggest that the perfluoro-15-crown-5-ether would be stably associated with a specific cell. In other words, there was no reason to think that perfluoro-15-crown-5-ether would be useful in methods for administering pre-labeled cells *in vivo*. Furthermore, the compositions of Schweighardt were not known to be safe and effective for subsequent administration of *ex vivo* labeled cells until the instant disclosure. The concentration of fluorocarbon and duration of exposure to a cell are different between *ex vivo* labeling and direct injection into a subject. One of ordinary skill in the art would not have expected fluorocarbon imaging reagents to be suitable for *ex vivo* labeling simply because they are suitable for direct injection. Thus, one of ordinary skill in the art would not have been motivated to combine the perfluoro-15-crown-5-ether of Schweighardt with the method of Phillips.

The remaining reference cited by the Examiner, Wilhelm, would not have motivated one of ordinary skill in the art to substitute the fluorocarbon imaging reagent of Schweighardt for the radionuclides of Phillips. For the reasons recited above, one of ordinary skill in the art would not have been motivated to label cells *ex vivo* with perfluoro 15-crown-5 ether. Wilhelm does not disclose perfluorocarbon imaging reagents, but rather relates to magnetic nanoparticles. Therefore, the ordinarily skilled worker would have had no reason to combine the method of Phillips with the imaging reagents of Schweighardt.

Accordingly, the present claims are not rendered obvious by the teachings of Phillips in view of Wilhelm and Schweighardt. Reconsideration and withdrawal of the rejection under 35 U.S.C. § 103(a) are respectfully requested.

Double Patenting Rejection

Claims 32, 45, 57 and 69-88 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 1-11, 15-18, 20, 25-27 and 28-30 of copending U.S. Application No. 11/787,521.

Applicant respectfully requests that the Examiner hold this rejection in abeyance until allowable subject matter is identified in the instant application. Once allowable subject matter has been identified, Applicant will evaluate the need for filing of a terminal disclaimer or providing arguments in view of the claims pending at that time.

CONCLUSION

Applicant believes no fee is due with this response in addition to the fees provided for on the Fee Transmittal sheet. However, if a fee is due, please charge our Deposit Account No. 18-1945, under Order No. CAMU-P01-002 from which the undersigned is authorized to draw.

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Respectfully submitted,

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